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Vascular endothelial growth factor before and after locoregional treatment and its relation to treatment response in hepatocelluar carcinoma patients

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ABSTRACT

Objective: To evaluate vascular endothelial growth factor (VEGF) levels in hepatocellular carcinoma patients before and after transcatheter arterial chemoembolization (TACE) and percutaneous ethanol injection (PEI) and its relation to treatment response.
Methods: A total of 40 patients with unrespectable hepatocellular carcinoma were assessed clinically. Twenty patients were suitable to be treated by TACE, while other 20 patients were treated with PEI. Serum VEGF levels were measured before and 1 month after each procedure by ELISA. Response was assessed after 1 month according to Union Internationale Contre le Cancer evaluation criteria based on change in tumor size as measured by ultrasound.
Results: There was no significant difference between TACE and PEI groups with regard to age, sex, tumor size, response to local therapy, or VEGF and alpha-fetoprotein before and after therapy. VEGF levels after TACE were significantly higher than before TACE [(298.1 ± 123.6) pg/mL *vs.* (205.8 ± 307.3) pg/mL; $P = 0.001$]. Also, VEGF levels were significantly higher after PEI than before PEI [(333.8 ± 365.6) pg/mL *vs.* (245.3 ± 301.8) pg/mL; $P = 0.000$]. Non-responders of both groups had significantly high VEGF levels than responder's, both before [(985.0 ± 113.2) pg/mL *vs.* (117.1 ± 75.3) pg/mL; $P < 0.001$] and after therapy [(1330.6 ± 495.7) pg/mL *vs.* (171.0 ± 94.7) pg/mL; $P = 0.000$)].
Conclusions: Both TACE and PEI were associated with an increase in serum VEGF in hepatocelluar carcinoma patients. Higher levels of VEGF before and after therapy were found in non-responders, suggesting that VEGF is a useful marker in predicting treatment response.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide and it is the 3rd common cause of cancer-related death [1]. In Egypt, with high prevalence of hepatitis C virus (HCV), HCC is reported as the most common cancer among males [2]. Although surgical resection and liver transplantation are the curative treatment for HCC, these options are usually limited due to poor surgical fitness,

inoperable lesion and shortage of liver donors [3]. Many of current non-surgical interventions improved survival and provided effective bridging therapy for liver transplantation [4]. Although radiofrequency ablation (RFA) is the first choice procedure for HCC treatment, percutaneous ethanol injection (PEI) is still a valuable option for small uninodular HCC especially in sites where thermal ablation is risky [3,5]. In addition, PEI is a simple, safe, effective, and cheap treatment with low complication rate [6]. Transcatheter arterial chemoembolization (TACE) is a safe procedure with a morbidity of less than 5% and mortality of 0.6% [7]. TACE alone or with other procedures as adjuvant therapy or before surgical treatment is currently used in patients with multinodular HCC, without vascular invasion or extrahepatic spread and well preserved liver function [8]. Vascular

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endothelial growth factor (VEGF) is one of the most potent angiogenic factors, which has been reported to be correlated with tumor metastasis, aggressiveness and poor prognosis in patients with HCC [9,10]. Recently, some studies demonstrated changes in VEGF after some locoregional therapies [11,12]. However, the relation of these changes to efficacy of therapy needs further evaluation. This study was conducted to evaluate VEGF level in HCC patients before and after TACE and PEI and its relation to treatment response.

2. Materials and methods

2.1. Study population

A total of 40 patients with HCC were included in this prospective study who were presented to National Cancer Institute and Internal Medicine Department of Kasr-Al Ainy Hospital in the period between September 2010 and February 2011. Enrollment criteria were: (1) absence of previous treatment for HCC, (2) unidimensionally and/or bidimensionally measurable Okuda stage I/II tumors, (3) pathologically proven lesion and (4) patients between 18 and 70 years of age and have Eastern Cooperative Oncology Group performance score 0–2 and an anticipated life expectancy of at least 8 weeks [13]. Twenty patients were eligible to PEI based on following criteria of their lesions: (1) less than 3 lesions, (2) well defined, (3) capsulated and (4) not near to liver surface. Another 20 patients were candidates for TACE with following selection criteria: (1) patency of portal vein, (2) absence of extrahepatic metastasis and (3) stage A or B Child-Pugh classification. Prior written informed consent was obtained from each patient and approval of local ethical committee was given before starting the study. All patients were evaluated clinically and with complete blood count, coagulation profile, and liver function. Disease stage was determined based on Union Internationale Contre le Cancer (UICC)/American Joint Committee on Cancer (AJCC) staging system [14]. Serum VEGF and serum alpha-fetoprotein (AFP) were measured before and 1 month after the procedures. Response to therapy was defined by standard UICC criteria based on the change in tumor size as assessed by ultrasound 1 month after the procedure. Collectively, patients who had complete remission, partial remission or stable disease were considered as responders, while those with progressive disease were considered as non-responders.

2.2. TACE procedure

An arterial catheter was inserted into the femoral artery by Seldinger method and placed in the hepatic artery. Tumor-feeding vessels were super-selected as possible and the catheter was inserted to the level of the segmental arteries, subsegmental arteries or lobar branches. A solution containing 50 mg of doxorubicin hydrochloride and 10 mL of ionized oil (lipidol) was infused through the catheter (5 French) or microcatheter (2.8 or 3 French).

2.3. PEI procedure

Under ultrasound guidance and after local anesthesia, a 22 gauge percutaneous transhepatic cholangiogram needle was inserted into the tumor. Absolute (99.5%) ethanol was injected at a dose of 2–10 mL.

2.4. Assay of serum VEGF level

Serum VEGF concentrations were quantitatively measured using ELISA kit (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, Minnesota, USA) according to manufacturer's instructions.

2.5. Statistical analysis

Data were analyzed using SPSS 17.0 statistical software. Numerical data were expressed as mean ± SD or median and ranged as appropriate. Qualitative data were expressed as frequency and percentage. *Chi*-square test was used to examine the relation between qualitative variables. For quantitative data, comparison between 2 groups was done using Mann–Whitney test or *t*-test. Comparison between 3 groups was done using Kruskal–Wallis test then *post-hoc* "Scheffe test" on the rank of variables was used for pair-wise comparison. Comparison of two repeated measures was done using Wilcoxon signed-rank test. A *P*-value < 0.05 was considered significant.

3. Results

3.1. Baseline clinicopathological characteristics

As shown in Table 1, patients included 34 males (85.0%) and 6 females (15.0%); age ranged from 54 to 69 years with a median of 61 years. Thirty-two patients were child A (80.0%) and 8 were child B (20.0%) classification. HCC etiology was related to HCV in 33 patients (82.5%) and hepatitis B virus in 7 patients (17.5%). According to UICC/AJCC staging system, 13 patients (32.5%) were stage IIIA and 27 patients (67.5%) were stage IIIB.

3.2. Clinical and pathological variables in TACE group compared to PEI

Comparison of clinical and pathological variables in TACE and PEI groups was presented in Table 2. There was no statistically significant difference between the 2 groups regarding age, sex, virology, Child-Pugh class, tumor size, AFP levels before (AFP-B), AFP after treatment, VEGF before (VEGF-B) or after (VEGF-A) therapy or response to local treatment.

Table 1

Baseline clinicopathological characteristics of 40 patients with HCC.

Characteristics		*n* (%)
Age (years)	< 60	20 (50.0)
	> 60	20 (50.0)
Sex	Male	34 (85.0)
	Female	6 (15.0)
Virology	HCV	33 (82.5)
Child-Pugh class	A	32 (80.0)
	B	8 (20.0)
UICC/AJCC staging	IIIA	13 (32.5)
	IIIB	27 (67.5)
Performance status	1	13 (32.5)
	2	27 (67.5)
Tumor size (cm)	2–5	32 (80.0)
	> 5	8 (20.0)
AFP (ng/mL)	< 400	31 (77.5)
	> 400	9 (22.5)

Table 2
Clinical and laboratory variables in TACE group compared to PEI group (*n* = 20). *n* (%).

Group	Age (years)		Sex		HCV positive	Child-Pugh class		Tumor size (> 5 cm)		AFP (> 400 ng/mL)		VEGF (pg/mL) (mean ± SD)		Responders
	< 60	> 60	Male	Female		A	B	Before therapy	After therapy	Before therapy	After therapy	Before therapy	After therapy	
TACE group	11 (55)	9 (45)	16 (80)	4 (20)	16 (80)	16 (80)	4 (20)	5 (25)	3 (15)	5 (25)	5 (25)	205.8 ± 307.3	298.1 ± 488.9	18 (90)
PEI group	9 (45)	11 (55)	18 (90)	2 (10)	17 (85)	16 (80)	4 (20)	3 (15)	3 (15)	4 (20)	4 (20)	245.3 ± 301.8	333.8 ± 365.6	17 (85)
P-value	0.53		0.66		1.00	1.00		0.69	1.00	1.00	1.00	0.13	0.15	1.00

3.3. Comparison of clinicopathological variables between responders and non-responders

There was no statistically significant difference between responders and non-responders regarding age, virology, tumor stage, Child-Pugh class and type of local therapy or response. About 80% of non-responders had initial tumor size > 5 cm compared to only 11.4% of responders (*P* = 0.003). All non-responders had AFP levels > 400 ng/mL compared to 11.4% of responders (Table 3).

3.4. VEGF levels in responders compared to non-responders

VEGF-B values were significantly higher in non-responders compared to responders [(985.0 ± 113.2) pg/mL *vs.* (117.1 ± 75.3) pg/mL; *P* < 0.001] (Table 3). Also, VEGF-A levels were significantly higher in non-responders than responders [(1 330.6 ± 475.9) *vs.* (171.0 ± 94.7) pg/mL; *P* = 0.000] (Table 3).

3.5. Comparison between pre and post-procedures of tumor size, AFP, VEGF in TACE and PEI groups

In TACE group, after the procedure the tumor size was statistically significant smaller and VEGF levels were significantly higher than before the procedure (*P* = 0.023, *P* = 0.001, by Wilcoxon–Mann–Whitney test). Also, VEGF significantly increased after PEI (*P* = 0.000, Wilcoxon–Mann–Whitney test). There was no significant change in AFP levels after both procedures (Table 4).

3.6. Relation of VEGF to tumor size and AFP

In TACE group, mean level of VEGF-B was significantly higher in patients with basal AFP > 400 ng/mL than in patients with AFP < 400 ng/mL (420 *vs.* 74.1 pg/mL; *P* = 0.001). Also, mean VEGF-A was significantly higher in patients with basal AFP > 400 ng/mL than in patients with AFP < 400 ng/mL (389 *vs.* 115 pg/mL; *P* = 0.015). No significant difference in VEGF-B

Table 3
Comparison of clinicopathological variables between responders and non-responders. *n* (%).

Group	Age (years)		Sex		HCV positive	Child-Pugh class		Tumor stage		Tumor size (> 5 cm)		AFP (> 400 ng/mL)		VEGF (pg/mL) (mean ± SD)		Type of therapy	
	< 60	> 60	Male	Female		A	B	IIIA	IIIB	Before therapy	After therapy	Before therapy	After therapy	Before therapy	After therapy	TACE	PEI
Responders (*n* = 35)	16 (45.7)	19 (54.3)	32 (91.4)	3 (8.6)	28 (80.0)	28 (80.0)	7 (20.0)	12 (34.3)	23 (65.7)	4 (11.4)	2 (5.7)	4 (11.4)	4 (11.4)	117.1 ± 75.3	171.0 ± 94.7	18 (51.4)	17 (48.6)
Non-responders (*n* = 5)	4 (80.0)	1 (20.0)	2 (40.0)	3 (60.0)	5 (100.0)	4 (80.0)	1 (20.0)	1 (20.0)	4 (80.0)	4 (80.0)	4 (80.0)	5 (100.0)	5 (100.0)	985.0 ± 113.2	1330.6 ± 475.9	2 (40.0)	3 (60.0)
P-value	0.340		0.010		0.560	1.000		1.000		0.003	0.001	0.001	0.001	< 0.001	0.000	1.000	

Table 4
Tumor size, AFP, VEGF pre and post-procedures. *n* (%).

Local therapy	Variable		Pre-procedure	Post-procedure	*P*-value[a]
Chemoembolization	Tumor size	2–5 cm	15 (75)	17 (85)	0.023
		> 5 cm	5 (25)	3 (15)	
	AFP	< 400 ng/mL	15 (75)	15 (75)	0.116
		> 400 ng/mL	5 (25)	5 (25)	
	VEGF (mean ± SD)		205.8 ± 307.3	298.1 ± 123.6	0.001
Ethanol injection	Tumor size	2–5 cm	17 (85)	17 (85)	0.203
		> 5 cm	3 (15)	3 (15)	
	AFP-B	< 400 ng/mL	16 (80)	16 (80)	0.431
		> 400 ng/mL	4 (20)	4 (20)	
	VEGF (mean ± SD)		245.3 ± 301.8	333.8 ± 365.6	0.000

[a]: *P*-values were evaluated using Wilcoxon–Mann–Whitney test.

Table 5

Relationships of VEGF levels and tumor size and AFP in TACE group.

Characteristics		Number of patients	VEGF-B (pg/mL) (mean range)	*P*-value	VEGF-A (pg/mL) (mean range)	*P*-value
Tumor size-B (cm)	2–5	15	77.0 (37.4–420.0)	0.349	132.1 (58.0–389.0)	0.349
	> 5	5	80.4 (57.6–1155.0)		115.2 (95.2–2165.3)	
AFP-B (ng/mL)	< 400	15	74.1 (37.4–198.0)	0.001	115.0 (58.0–345.2)	0.015
	> 400	5	420.0 (130.0–1155.0)		389.0 (100.0–2165.3)	

or VEGF-A in patients with basal tumor size > 5 cm compared to patients with tumor size 2–5 cm (Table 5).

4. Discussion

Assessment of tumor response to locoregional therapy is important and can improve survival in HCC patients. Current evaluation of treatment response depends on radiological assessment, although biological changes may be more informative and earlier than anatomical changes. So, biochemical markers are demanding for prediction of treatment response. High pretreatment VEGF was found to predict poor response and survival in patients undergoing TACE for HCC [15,16]. Same results have been found in another study in HCC patients undergoing RFA [17]. To our knowledge, there is no previous reports in patients receiving PEI. We found that pretreatment VEGF levels were significantly higher in the non-responders of both TACE and PEI groups compared to responders. VEGF activates intracellular receptor kinases which result in tumor growth and new vessel formation leading to more aggressive tumor with poor prognosis [17]. Although previous studies found that VEGF increased shortly after TACE with peak levels during first post week and slow decrease thereafter [11,18], our data demonstrated that VEGF was significantly increased 1 month after both TACE and PEI and levels were significantly higher in non-responders than responders. This goes with the results of one study that VEGF levels were significantly higher in non-responders at 4 weeks after TACE [19]. Moreover, higher tissue levels of VEGF were also reported in HCC specimens from patients who received TACE than those who didn't [20]. Another study found significant increase in microvascularity after TACE in HCC patients [21]. The changes in VEGF levels after both TACE and PEI may be caused by tissue hypoxia resulted from tissue damage induced by therapy. Hypoxia inducible factor has been found to increase transcriptional activity of serum VEGF [22]. This increase in VEGF may help the survival of residual tumor cells [23]. Similar findings were reported in a recent study of HCC patients who underwent RFA [12]. This could suggest that changes in VEGF after locoregional interventions are related to the degree of tissue necrosis caused by treatment irrespective of the therapeutic modalities.

Although many studies reported the prognostic significance of AFP in the outcome of HCC after locoregional therapies [24,25], others have shown its poor detection rate of small residual tumor size after treatment [26]. In the current study, AFP was significantly higher in non-responders than responders but there was no significant difference in AFP-B and after TACE or PEI. This may be related to its long half-life which interferes with significant changes in its level after therapy. In our study, VEGF levels were significantly higher in patients with baseline AFP > 400 ng/mL. This coincides with what was reported that AFP is a pro-angiogenesis factor, possibly in a VEGF dependent

manner [27]. This can be explained by two recent findings: first, AFP concentration had significant correlation with increased VEGF-A expression in HCC cells [28]; second, silencing of AFP expression significantly reduced the expression levels of VEGF [29]. From previously mentioned, VEGF may be a better marker than AFP for early evaluation of treatment response.

Although in our study responders had significantly higher tumor size than non-responders, no significant difference of VEGF level was found between patients with tumor size > 5 cm and those with smaller size. This result seems to contradict one study reported that tumors measuring > 2 cm had higher VEGF levels than those < 2 cm [30]. This difference may be related to a higher cut of point in our study. The current study found significant increase in VEGF after both PEI and TACE.

In addition, non-responders had higher levels of VEGF than responders. These results suggest VEGF as possible biochemical marker in HCC patients receiving TACE or PEI and may help in the selection of patients who need adjuvant therapy. It is worth to mention that our study has some limitations such as mall sample size and follow up measurement of VEGF.

Conflict of interest statement

We declare that we have no conflict of interest.

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